J. Helminthol. Soc. Wash.
59(2), 1992, pp. 149–152

Can Autoinfection be Provoked in the *Strongyloides ratti*-infected Gerbil, *Meriones unguiculatus*?

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ABSTRACT: *Strongyloides ratti* infections in gerbils, *Meriones unguiculatus,* are chronic when compared to infections with this species in other rodent hosts. Furthermore, eggs are rarely found in freshly passed feces, instead, resembling *Strongyloides stercoralis* infection, rhabditiform, first-stage larvae are characteristically expelled. The longevity of *S. ratti* infection in gerbils is not, however, due to naturally occurring autoinfection, nor could autoinfection be induced by immunosuppression or prolongation of intestinal transit time.

KEY WORDS: nematode, *Strongyloides ratti,* gerbil, *Meriones unguiculatus,* autoinfection, prednisolone acetate, diphenoxylate.

Experimental murine strongyloidiasis, involving *Strongyloides ratti* and the rat, is a frequently used laboratory model for human strongyloidiasis. Unfortunately, however, this host–parasite association does not mimic the 2 most characteristic features of *Strongyloides stercoralis* infection in man, namely autoinfection and the unusual propensity to establish extremely chronic infections. Indeed, several attempts to provoke autoinfection in *S. ratti*-infected rodents have failed and adult worms are expelled from the usual rodent hosts (rats and mice) within the first 2 to 3 wk of infection (Dawkins, 1989). In this connection, it is interesting that an isolate of *Strongyloides* (probably *S. ratti*) from a muskrat survived in gerbils for a year (M. D. Little, pers. comm.).

Our own experience with a well-known strain of *S. ratti* indicates that infections in gerbils remain patent for at least 5 mo. This longevity of infection in gerbils, as well as our observation that in this host the eggs of *S. ratti* almost invariably hatch internally, prompted the 2 major questions addressed in this study: 1. Could the unusual longevity of a primary infection be due to naturally occurring autoinfection? and 2. Because the eggs hatch internally, could autoinfection be induced (or enhanced) by either increasing intestinal transit time and/or depressing the host's immune responses?

Materials and Methods

Parasite strain

Strongyloides ratti infective third-stage larvae (L_3) were obtained from fecal cultures of donor rats maintained in our laboratory. The strain (G60, a heterogonic strain) was obtained originally from Dr. M. E. Viney, University of Edinburgh, Edinburgh, Scotland.

Experimental animals

Purpose-bred female gerbils were purchased from Tumblebrook Farm, Inc. (West Brookfield, Massachusetts), housed 5 to a cage, and fed Purina Lab Chow and water ad libitum.

Experimental designs

To confirm that *S. ratti* infections in gerbils are in fact long-lasting, 2 4–6-week-old animals were injected subcutaneously (SQ) with 500 and 700 L_3, respectively. The number of L_1's shed in the feces was determined weekly using the McMaster technique, which has a lower detection limit of 100 larvae/g. A third gerbil received 1,000 L_3 SQ and 3 mg of prednisolone acetate on 4 alternate days beginning on the day of infection.

For an experiment designed to provoke (or enhance) autoinfection by immunosuppression, increasing the intestinal transit time, or by both treatments combined, 40 gerbils were injected with 1,000 L_3 *S. ratti* SQ. The infected animals were divided into 4 groups of 10 each: infected controls, prednisolone-treated, diphenoxylate-treated, and a group treated with both drugs.

Prednisolone acetate (3 mg/gerbil on alternate days for 4 treatments and then at 4-day intervals for the duration of the experiment) was selected as the immunosuppressive agent of choice, Kamiya and Sato (1990) having used it successfully to adapt the complete life cycle of *Echinococcus multilocularis* to the gerbil, a normally nonpermissive definitive host. To increase intestinal transit time, the motility-altering, antidiarrheal drug diphenoxylate was used at the dosage suggested by Megens et al., 1989 (8.15 mg/kg per os) on alternate days.

The animals in each group were divided into 2 lots of 5, 1 scheduled for euthanasia at about patency (days 6–8) and the other day 21, when infections normally have been expelled from rats and mice. These 2 necropsy times were based on unpublished observations and theoretical considerations (Schad, 1989) suggesting that autoinfection, it it were to occur, would maximize early in the infection (i.e., about the time of patency), and if it did extend the infection beyond its normal duration in rodents, would have to occur before the infection is normally expelled.

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Table 1. Recovery of first-stage larvae of *Strongyloides ratti* from the intestines of gerbils at 2 times postinfection.

Group	Number of larvae recovered (geometric mean)*†	
	Day 6–8	Day 21
Infected controls	3,197.1	134.6[a]
Prednisolone-treated	4,187.1	332.6
Diphenoxylate-treated	9,319.8	103.6[b]
Prednisolone- and diphenoxylate-treated	458.4	854.1

* Results of dilution counts.

† Except as indicated by superscript letters, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 infected controls (Mann-Whitney *U*-test). [b] = Significantly different ($P = 0.04$) from day 6–8 diphenoxylate-treated group (Mann-Whitney *U*-test).

On the day of worm recovery, each gerbil was euthanatized and its intestines removed. The small intestine was slit longitudinally, rinsed of contents, and hung in a 50-ml centrifuge tube of phosphate-buffered saline (PBS). Its contents were suspended in a separate tube of PBS in which they were allowed to sediment for 30 minutes, at which time differential dilution counts were made of all life history stages represented. The cecum, large intestine, and rectum were also slit longitudinally and hung in a tube of PBS. Their contents were Baermannized at 37°C for 1 hr and any organisms recovered counted as above. The slit small and large intestines were incubated at 37°C for 3 hr when they were transferred to another tube of PBS for an additional 3 hr. After removal of the intestines, the suspended material in these tubes was allowed to sediment and parasites were counted as above.

Parasite fecundity was estimated in groups of hosts not given diphenoxylate by dividing the total number of larvae found in the intestines by the number of adult worms recovered.

Table 2. Recovery of adult *Strongyloides ratti* from the intestines of gerbils at 2 times postinfection.

Group	Number of adults recovered (geometric mean)*†	
	Day 6–8	Day 21
Controls	222.6	36.0
Prednisolone-treated	148.4	53.6
Diphenoxylate-treated	319.5	35.6[a]
Prednisolone- and diphenoxylate-treated	51.5	81.3

* Results of dilution counts.

† Except as indicated by the superscript letter, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 diphenoxylate-treated group (Mann-Whitney *U*-test).

Table 3. Fecundity of *Strongyloides ratti* adults in normal and immunodepressed gerbils.

Day post-infection	Group	Number of larvae recovered/ number of adults recovered*
6–8	Infected controls	14.3
	Prednisolone-treated	18.5
21	Infected controls	5.2[a]
	Prednisolone-treated	10.4

* Geometric mean (results of dilution counts). Except as indicated by the superscript letter, there were no statistically significant differences between groups. [a] = Significantly different ($P = 0.05$) from day 6–8 infected controls (Mann-Whitney *U*-test).

Statistical analysis

There was excessive mortality in the experimentally treated groups, so that 3 animals, rather than the statistically preferred 5 animals originally anticipated, were available for worm counts at 1 wk after infection. Only 4 and 3 animals, respectively, survived in the diphenoxylate and the combined treatment groups, at 3 wk after infection. Given the highly variable nature of worm count data and the small group sizes, geometric mean worm counts formed the basis for all statistical comparisons. All data (Tables 1–3) are presented as geometric means based on the ln(count + 1).

Results

The course of each of 3 *Strongyloides ratti* infections in gerbils is shown in Figure 1. The gerbil receiving the lowest larval dose remained positive by McMaster count for 22 wk and had no worms in its intestines when necropsied at 25 wk postinfection (PI). The second gerbil, last positive by McMaster at 24 wk PI, was still positive by coproculture, a more sensitive test, at 44 wk PI. The gerbil given 1,000 L_3 and initially treated with prednisolone maintained a higher level of larval output than either of the normal gerbils (Fig. 1) and at 29 weeks (the time at which this report was written) still had >100 L_1/g of feces. Other preliminary experiments (data not shown) indicated that doses exceeding 1,000 *S. ratti* L_3 were fatal in 4–5-week-old gerbils. *Strongyloides ratti* infective third-stage larvae obtained from coprocultures of the gerbil that received 1,000 L_3 were infective to mice and produced infections that were similar to those initiated by L_3's from rats. A gerbil that received 1,000 L_3's similarly derived has shown a pattern of larval shedding in the feces similar to the original 1,000 L_3 gerbil for 1 mo, the time interval to date (data not shown).

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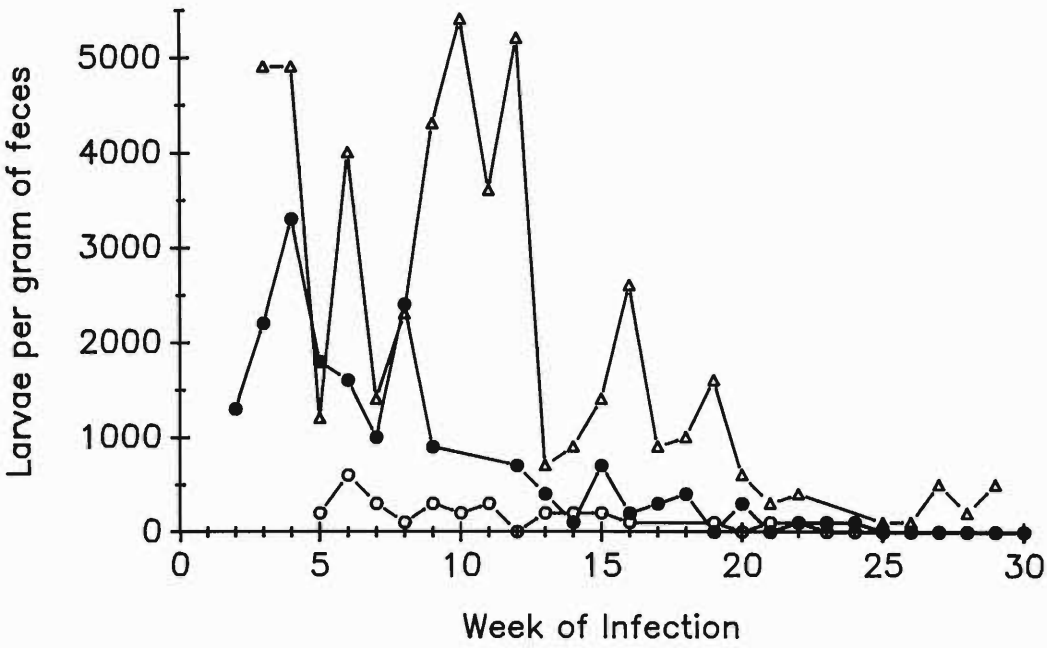

Figure 1. Course of infection of *Strongyloides ratti* in the gerbil as measured by the number of larvae in the feces. Gerbils were infected subcutaneously with 500 L_3's (third-stage larvae) (○——○), 700 L_3's (●——●), or 1,000 L_3's (△——△).

In groups of gerbils treated with prednisolone and/or diphenoxylate, *no* third-stage autoinfective larvae were found at either time period; only eggs , L_1's, and adult worms were recovered. Periodic weighing of the feces from the cages (5 gerbils/cage) of diphenoxylate-treated and untreated gerbils indicated that the treated gerbils produced less feces than untreated gerbils. The stage-specific parasite recoveries for the time of patency (1 wk) in the 3 experimentally treated groups were compared to those of the infected control group; none of the former differed significantly (Mann-Whitney *U*-test) from those of the latter (Tables 1, 2). Similarly, there were no statistically significant differences (Mann-Whitney *U*-test) between the worm populations of the experimentally treated groups necropsied after 3 wk and those of the control group (see Tables 1, 2). However, in most groups there appeared to be a marked decrease in the number of worms (both larvae and adults) between 1 and 3 wk postinfection, but given the small group sizes and the variability of the data, this decrease was significant in only 2 of 4 and 1 of 4 possible comparisons (see Tables 1 and 2, respectively).

There was excessive mortality of gerbils (7 of 29) in the diphenoxylate-treated groups, perhaps reflecting the relatively high parasite dose, approaching that fatal for gerbils, in interaction with a regimen of drug use designed for the rat.

Fecundity of *S. ratti* was compared in groups that were not treated with diphenoxylate. At 1 and 3 wk PI, there was a decrease in fecundity of border-line significance ($P = 0.05$) in the infected control group with only 36% of the week 1 larval output occurring at 3 wk. In contrast, there was no statistically significant decrease in the fecundity of worms between 1 and 3 wk PI in immunodepressed gerbils.

Discussion

Prompted by observations indicating that gerbils harbor unusually chronic *Strongyloides ratti* infections, we sought to determine whether this chronicity reflects an underlying autoinfection occurring in the *S. ratti*-infected gerbil. Our investigations were designed to determine if autoinfection occurs naturally in *S. ratti*-infected gerbils or if it could be induced (or enhanced) with immunosuppressive agents and/or intestinal motility-altering drugs. Treatment with immunosuppressive drugs, particularly corticosteroids such as prednisolone, is well known to increase autoinfection in *Strongyloides stercora-*

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lis infections (Grove et al., 1983; Schad et al., 1984; Genta et al., 1986). Similarly, opium, a drug that decreases intestinal motility and, therefore, increases intestinal transit time increases autoinfection in experimental canine strongyloidiasis (Nishigori, 1928). However, no autoinfective larvae (L_3) were found in either the small or large intestines of the gerbils at either of 2 critical time points in any of the treatment groups, suggesting that autoinfection is not responsible for the prolongation of *S. ratti* infection in gerbils beyond the 3-wk interval of patency that normally occurs in other rodents. Under the conditions of this experiment, prednisolone acetate and diphenoxylate had no demonstrable effect on the number of L_1 or adult worms recovered. This may, however, be attributable to the marked variability in worm counts and the small sample size.

In contrast to the short infections in rats induced by infection with the usual large numbers of larvae, Graham (1940) was able to establish chronic infections of *S. ratti* in rats by infecting them with 1 larva. In these rats, the infection lasted for an average of 149 ± 8 days with a range of 8–477 days. Graham's results suggest that there may be a minimum antigenic load needed to cause the expulsion of the adult worms as there was no indication that autoinfection was maintaining the worm burden. A similar mechanism may be operating in the gerbil, allowing a small number of worms that survive the initial expulsion to remain without provoking a further immune response from the gerbil.

The exceedingly chronic *S. ratti* infections occurring in the gerbil more closely resemble human *S. stercoralis* infections than do *S. ratti* infections in rats and mice. Consequently, even though autoinfection does not occur in *S. ratti*-infected gerbils, this host–parasite association should prove useful for the experimental investigation of human strongyloidiasis. Furthermore, the chronicity of the infection in the gerbil makes it an excellent host for maintaining *S. ratti* in the laboratory. The postponement of the necessity to transfer the infection from 2 wk, as is the case in rats, to 5 mo (or even longer) saves animals and reduces the cost of strain maintenance substantially.

Acknowledgments

This work was supported by NIH Grant 5RO1-AI-22662. We thank 2 anonymous reviewers for their comments, which contributed significantly to the completeness of our discussion.

Literature Cited

Dawkins, H. J. S. 1989. *Strongyloides ratti* infections in rodents: value and limitations as a model of human strongyloidiasis. Pages 287–332 *in* D. I. Grove, ed. Strongyloidiasis: A Major Roundworm Infection in Man. Taylor & Francis, New York.

Genta, R., G. A. Schad, and M. Hellman. 1986. *Strongyloides stercoralis*: parasitological, immunological and pathological observations in immunosuppressed dogs. Transactions of the Royal Society of Tropical Medicine and Hygiene 80:34–41.

Graham, G. L. 1940. Studies on *Strongyloides* VII. Length of reproductive life in a homogonic line of singly established *S. ratti*. Revista de Medicina Tropical y Parasitologia 4:89–104.

Grove, D. I., P. J. Heenan, and C. Northern. 1983. Persistent and disseminated infections with *Strongyloides stercoralis* in immunosuppressed dogs. International Journal of Parasitology 13:483–490.

Kamiya, M., and H. Sato. 1990. Complete life cycle of the canid tapeworm, *Echinococcus multilocularis*, in laboratory rodents. The FASEB Journal 4:3334–3339.

Megens, A., L. Canters, F. Awouters, and C. Neimegeers. 1989. Is in vivo dissociation between the antipropulsive and antidiarrheal properties of opioids in rats related to gut selectivity? Archives Internationales de Pharmacodynamie et de Therapie 298:220–229.

Nishigori, M. 1928. On various factors influencing the development of *Strongyloides stercoralis* and autoinfection. Taiwan Igakkai Zassi 27:1–56. (Extracted and translated from Japanese. Pages 340–345 *in* B. Kean, K. E. Mott, and A. J. Russell, eds. Tropical Medicine and Parasitology, Classic Investigations. Vol. 2. 1978. Cornell University Press, Ithaca, New York.)

Schad, G. A. 1989. Morphology and life history of *Strongyloides stercoralis*. Pages 85–104 *in* D. I. Grove, ed. Strongyloidiasis: A Major Roundworm Infection in Man. Taylor & Francis, New York.

———, **M. Hellman, and D. W. Muncey.** 1984. *Strongyloides stercoralis*: hyperinfection in immunosuppressed dogs. Experimental Parasitology 57:287–296.

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